model is suggestive, it remains to be shown whether TRADD plays the role of a key regulator in this process. This could be addressed by using TRADD mutants with selective deletion of the FADD and RIP/TRAF2 binding sites. The role of other signals emanating from the TNFR1 in this process remains to be examined; for example, the engagement of RAIDD/caspase-2 and the activation of the JNK and MAPK pathways by TNFR1 have not been placed in the context of these findings. Additionally, the nature of the protein that is responsible for the posttranslational modifications of the TNFR1 signaling components has not been addressed. Many pathological situations are characterized by an imbalance between NF-κB-regulated survival and caspase-mediated apoptosis signals. The identification of two distinct complexes regulating these two processes could now provide the means for their selective targeting for therapeutic purposes to treat diseases such as cancer and autoimmunity.

Bryan C. Barnhart and Marcus E. Peter
The Ben May Institute for Cancer Research
University of Chicago
924 East 57th Street
Chicago, Illinois 60637

Selected Reading

Barnhart, B.C., Alappat, E., and Peter, M.E. (2003). Sem. Immunol. *15*, 185–193.

Harper, N., Hughes, M., MacFarlane, M., and Cohen, G.M. (2003). J. Biol. Chem. *278*, 25534–25541.

Lee, J.C., and Peter, M.E. (2003). Immunol. Rev. *193*, 39–47.

Legler, D.F., Micheau, O., Doucey, M.A., Tschopp, J., and Bron, C. (2003). Immunity *18*, 655–664.

Micheau, O., and Tschopp, J. (2003). Cell *114*, this issue, 181–190.

Peter, M.E., and Krammer, P.H. (2003). Cell Death Differ. *10*, 26–35.

Shu, H.B., Takeuchi, M., and Goeddel, D.V. (1996). Proc. Natl. Acad. Sci. USA *93*, 13973–13978.

Schutze, S., Machleidt, T., Adam, D., Schwandner, R., Wiegmann, K., Kruse, M.L., Heinrich, M., Wickel, M., and Kronke, M. (1999). J. Biol. Chem. *274*, 10203–10212.

Varfolomeev, E.E., Schuchmann, M., Luria, V., Chiannilkulchai, N., Beckmann, J.S., Mett, I.L., Rebrikov, D., Brodianski, V.M., Kemper, O.C., Kollet, O., et al. (1998). Immunity *9*, 267–276.

Yeh, W.C., Pompa, J.L., McCurrach, M.E., Shu, H.B., Elia, A.J., Shahinian, A., Ng, M., Wakeham, A., Khoo, W., Mitchell, K., et al. (1998). Science *279*, 1954–1958.

Gene Switching by Metabolic Enzymes—How Did You Get on the Invitation List?

Histone gene expression in mammalian cells is codependent upon the Oct-1 transcription factor and its cognate, OCA-S coactivator complex. Surprisingly, GADPH plays an essential role in the OCA-S complex and confers redox dependence upon the in vitro transcription of histone genes.

Biochemists and geneticists lust to discover new paradigms of biological regulation. Both camps apply their trade in crafty ways aimed at dissecting uncharted pathways ranging from intracellular signaling to cell division, embryonic patterning, or even animal behavior. Most fun of all is the discovery of a "pioneer" protein that can be assigned to a new chore. Landing on these pioneers used to be the norm, in each case offering the challenge of bootstrapping one's way from polymeric matter to function. As our body of biological knowledge has blossomed over the past 25 years, one of the best shortcuts to understanding detailed biochemical function—were that not part of the fundamental assay being employed—has been the use of the classic BLAST search. If X barked like Y, and if one had an inkling that Y might be of canine ancestry, then dog chow would be included on the supplies budget of the next grant application.

As our information base matures, fewer and fewer times do we end up with a virgin pioneer. Instead, one of two things now happens. We may find that the putative function of our "new" macromolecule has already been spoken for in some half-baked transient transfection assay, centered around some over-hyped field, sponsored by some top-down RFA, out of some "product-seeking" NIH institute, responsive to some congressional muscle, being lobbied by some special interest group, with the term "omics" almost always in close proximity. Alternatively, and much more interestingly, our new baby may have already been shown to perform a distinct function bolstered by hard-nosed biochemistry, biophysics, or genetics—as an example, an enzyme having an evolutionarily conserved role in intermediary metabolism.

What is one to think when a long and arduous genetic screen or biochemical purification leads to a protein firmly established to perform an entirely different function? The first time I remember this happening was back when Piatigorsky and colleagues discovered the recruitment of metabolic enzymes as lens crystallin proteins in the vertebrate eye. Lens crytallin proteins are related to, or are one-and-the-same as, argininosuccinate lyase, enolase, glutathione S-tranferase, and lactate dehydrogenase (Wistow and Piatigorsky, 1987). In this seminal case, one can safely conclude that the crystallin proteins do not link lens function to metabolism, nor are the enzymatic capabilities of the proteins actually operative in the focusing of light. Some other property of these enzymes, perhaps their shape and ability to aggregate in an ordered manner, must have filled the functional niche demanded of the vertebrate lens.

Zheng, Roeder, and Luo report in the current issue of *Cell* (Zheng et al., 2003) the biochemical purification and characterization of a transcriptional coactivator complex that contains, as an essential component, the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Is this a case of GAPDH acting out a surrogate role totally independent of its job in intermediary metabolism, analogous to the lens crystallin proteins? Or is its presence telling us something more intriguing—that the role of GAPDH in gene activation is either dependent upon its catalytic function, or influenced by the metabolic state of the cell? If so, an understanding of the traditional role of the enzyme might provide unanticipated insight into the process under study.

Let's start with the basics. For many years, the Roeder laboratory has been studying histone gene regulation. S-phase-dependent transcription of the mammalian gene encoding histone H2B requires a DNA binding protein designated Oct-1. In turn, Oct-1 alone cannot activate histone gene transcription, leading Zheng and colleagues to search for and identify a coactivator complex designated OCA-S. Purified OCA-S contains seven polypeptide constituents in the 300 kDa complex; remarkably, five of the seven polypeptides encode enzymes. The smallest p18 and p20 subunits of OCA-S were identified as nm23-H1 and nm23-H2 and have been claimed to specify nucleoside diphosphate kinase activity (Freije et al., 1998). One of the p36 doublet bands of the complex was identified as uracil-DNA glycosylase, with the other representing lactate dehydrogenase. The p38 component of OCA-S is indistinguishable from GAPDH. Finally, the larger p60 and p65 subunits were identified as the Sti1 and HSP70 chaperone proteins.

Among this diverse set of bedfellows, Zheng and colleagues focused most carefully on the p38/GAPDH subunit. This protein was shown to interact directly with the POU domain of Oct-1, contain intrinsic transactivation potential, be stringently essential for Oct-1-dependent transcription in vitro, and be recruited in an S-phase-specific manner to the promoter of the gene encoding histone H2B. Moreover, by use of RNAi-mediated gene silencing methods, the authors demonstrated the essential role of GAPDH for H2B transcription in cultured mammalian cells. Since GAPDH is essential for glycolysis, the authors needed to supplement their cultured cells with pyruvate in order to assess the role of this OCA-S subunit in histone gene transcription.

These various lines of experimentation provide compelling evidence that GAPDH is a critical component of the apparatus mammalian cells utilize to facilitate S-phase-specific H2B gene expression. This prompts two questions. First, is the enzymatic activity of GAPDH essential for its role as a transcriptional activator protein? Second, might the metabolic state of the cell feed back to influence the ability of GAPDH to act as a transcriptional coactivator?

The former of these questions has not been answered. GAPDH is an obligate homotetramer in its role as a cytosolic catalyst for the conversion of glyceraldehyde-3-phosphate (GAP) to 1,3-bisphosphoglycerate (1,3-BPG) during glycolysis. Since the purified OCA-S complex contains stoichiometric amounts of each of its subunits, it is therefore unlikely that GAPDH would form a homotetramer capable of this catabolic reaction.

Zheng and colleagues do, however, provide tantalizing evidence that several functional roles of GAPDH in its guise as a transcriptional coactivator are sensitive to the redox state of NAD. The potential importance of this observation requires a refreshed understanding of the reaction catalyzed by GAPDH during glycolysis. The first step of this elegant reaction involves condensation of the aldehyde moeity of GAP with the sulfhydryl group of an active site cysteine. A hydride ion is then transferred to a molecule of NAD^+ tightly bound and adjacent to the cysteine, leading to the release of NADH, and leaving a thioester intermediate. In the final step, orthophosphate attacks the thioester to form 1,3-BPG and regenerate a free cysteine. Remarkably, Zheng and colleagues show that the interaction between GAPDH and the Oct-1 POU domain is redox sensitive. NAD^+ significantly enhances this interaction in a dose-dependent manner. By contrast, NADH inhibits GAPDH binding to Oct-1. This observation was extended two steps further. First, the authors show that OCA-S interaction with the H2B promoter in a nuclear extract is enhanced by NAD^+ and inhibited by NADH. Second, in vitro transcription of the H2B promoter was found to be similarly responsive to the redox state of the NAD cofactor.

These represent nontrivial experimental accomplishments that were subjected to rigorous controls. Most convincingly, it was shown that a second Oct-1 coactivator, designated OCA-B, was insensitive to redox state whether assayed by measurements of direct interaction with the POU domain of Oct-1, or in vitro transcription. OCA-B, which abets the role of Oct-1 in immunoglobulin gene transcription, is not endowed with subunits stolen from intermediary metabolism (Luo and Roeder, 1999). At a minimum, these new observations indicate that histone gene transcription in vitro is sensitive to the redox state of bound NAD cofactor, and that this sensitivity is conferred by the OCA-S coactivator complex. It is also notable that OCA-S contains a single subunit of lactate dehydrogenase (LDH), yet another glycolytic enzyme. In contrast to GAPDH, LDH-mediated conversion of pyruvate to lactate results in the oxidation of NADH to NAD^+. Since the binding of both purified GAPDH and intact OCA-S to the POU domain of Oct-1 similarly favor an oxidized NAD^+:NADH redox ratio, it would appear that the redox sensing function of the co-activator is specified by GAPDH rather than LDH. Whether the OCA-S system is built to couple histone gene expression to the cellular redox state in living cells remains an unclear but exciting question.

This bold new work from our colleagues at Rockefeller University has the scent of several other studies percolating through the recent literature on eukaryotic gene expression. As an example, the Shi laboratory at Harvard Medical School recently reported the purification of a transcriptional corepressor complex designated CtBP (Shi et al., 2003). Analysis of the 20 or more subunits identified by mass spectrometry in this corepressor complex led to the prediction/confirmation of dehydrogenase, polyamine oxidase, and enoyl CoA isomerase/hydratase enzyme activities (not to mention multiple histone deacetylases and methylases). It has also been reported that CtBP binding to cellular and viral transcriptional repressor proteins is regulated in test tube reactions by nicotinamide adenine dinucleotide cofactors, and that this binding reaction is preferentially enhanced by reduced NADH compared with oxidized NAD^+ (Zhang et al., 2002). Functional studies of the CtBP complex are at a rudimentary level relative to the work highlighted herein on OCA-S. The techniques are, however, at hand to ask whether the enzymes present in the CtBP complex are acting out surrogate roles fully independent from their "day jobs"—or whether they might somehow deploy their inherent properties as metabolic enzymes as a means of fostering transcriptional repression depending on their sensing of metabolic state.

It is safe to bet that the OCA-S and CtBP complexes will not be alone in coopting enzymes formerly relegated to our Lehninger textbooks of biochemistry. It is also

exciting to consider the possibility that these enzymes may offer a coupling of gene expression to the metabolic state of the cell. With hindsight, one can rationalize why these discoveries are principally being driven by test tube biochemistry rather than classical forward genetics. Many of the enzymes present in the OCA-S and CtBP complexes are essential for cell viability, even in model eukaryotic organisms such as baker's yeast. As such, standard genetic approaches might have difficulty in nailing mutated alleles of genes encoding dual-use enzymes; or, at best, catch one component of a complex, as shown by the history of fruit fly genetic studies of CtBP (Perrimon et al., 1996).

It is fitting to close with an attempt to weave these exciting studies on OCA-S and CtBP with the obvious importance of linkages between metabolic state and gene regulation. Cells and organisms have evolved exceptional professional competence in properly coupling metabolism to gene expression. When cholesterol is in short supply, cells deploy a sophisticated means of sensing this shortage and mobilizing the latent SREBP transcription factor to activate the enzymatic apparatus required for de novo cholesterol biosynthesis (Horton et al., 2002). An abundance of carbohydrates is similarly sensed, allowing hepatocytes to mobilize the latent ChREBP transcription factor such that sugar can be efficiently converted to fat (Kabashima et al., 2003). Hard-nosed stories like this abound in the literature. They make total sense and were discovered by virtue of the rational expectation that metabolic homeostasis should take advantage of regulatory events controlling the expression of genes encoding metabolic enzymes.

This linkage between metabolism and gene regulation may, however, be far more extensive, baroque, and intricate than what can be anticipated from the rational approaches evolved from classical studies going back to Jacob and Monod. Admitting a bit of self-promotion, I close with observations reported from my own laboratory that are formally similar to work from the Roeder and Shi groups. In studies of the molecular pathway controlling circadian rhythm, we stumbled over potential connections with intermediary metabolism. Instead of finding enzymes associated with the system, we found heme directly associated with neuronal PAS domain protein 2 (NPAS2), a transcription factor essential for the control of circadian rhythm in the mammalian brain (Dioum et al., 2002). Paradoxically we likewise found that the DNA binding activity of this factor—at least in test tube reactions—is sensitive to the balance of reduced-to-oxidized NAD cofactors (Rutter et al., 2002). While we were not looking for any connection between metabolism and circadian rhythm, it is difficult to be blind to the fact that one's purified protein is red! This would be akin to ignoring the uninvited dinner guest sitting right across the table. As with the question of whether GAPDH is no more than a lens crystallin protein in the context of OCA-S, perhaps the presence of heme in NPAS2 is totally surrogate. If not already obvious, however, my bets are placed in the opposite corner.

Steve McKnight
Department of Biochemistry
University of Texas Southwestern Medical Center
5323 Harry Hines Boulevard
Dallas, Texas 75390

Selected Reading

Dioum, E.M., Rutter, J., Tuckerman, J.R., Gonzalez, G., Gilles-Gonzalez, M.-A., and McKnight, S.L. (2002). Science *298*, 2385–2387.

Freije, J.M., MacDonald, N.J., and Steeg, P.S. (1998). Biochem. Soc. Symp. *63*, 261–271.

Horton, J.D., Goldstein, J.L., and Brown, M.S. (2002). J. Clin. Invest. *109*, 1125–1131.

Kabashima, T., Kawaguchi, T., Wadzinski, B.E., and Uyeda, K. (2003). Proc. Natl. Acad. Sci. USA *100*, 5107–5112.

Luo, Y., and Roeder, R.G. (1999). Cold Spring Harb. Symp. Ouant. Biol. *63*, 119–131.

Perrimon, N., Lanjuin, A.C., Arnold, C., and Noll, E. (1996). Genetics *144*, 1681–1694.

Rutter, J., Reick, M., Wu, L.-J., and McKnight, S.L. (2002). Science *293*, 510–512.

Shi, Y., Sawada, J.-I., Sui, G., Affar, E.-B., Whetstine, J.R., Lan, F., Ogawa, H., Luke, M.P.-S., Nakatani, Y., and Shi, Y. (2003). Nature *422*, 735–738.

Wistow, G., and Piatigorsky, J. (1987). Science *236*, 1554–1556.

Zhang, Q., Piston, D.W., and Goodman, R.H. (2002). Science *295*, 1895–1897.

Zheng, L., Roeder, R.G., and Luo, Y. (2003). Cell *114*, this issue, 255–266.